T-bet controls regulatory T cell homeostasis and function during type-1 inflammation

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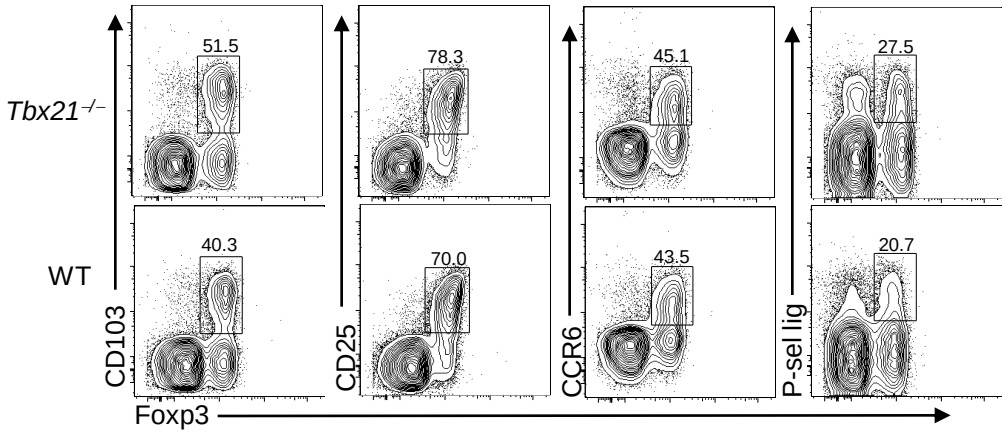
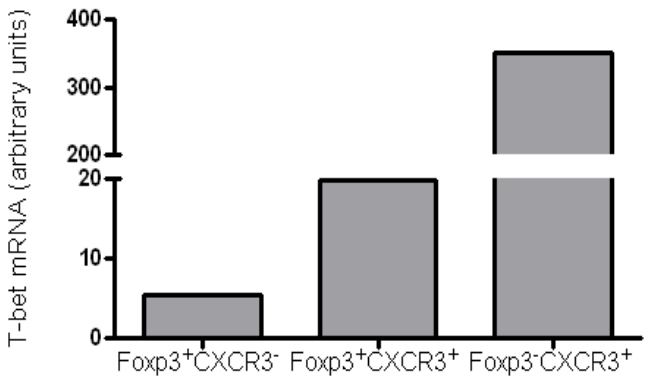
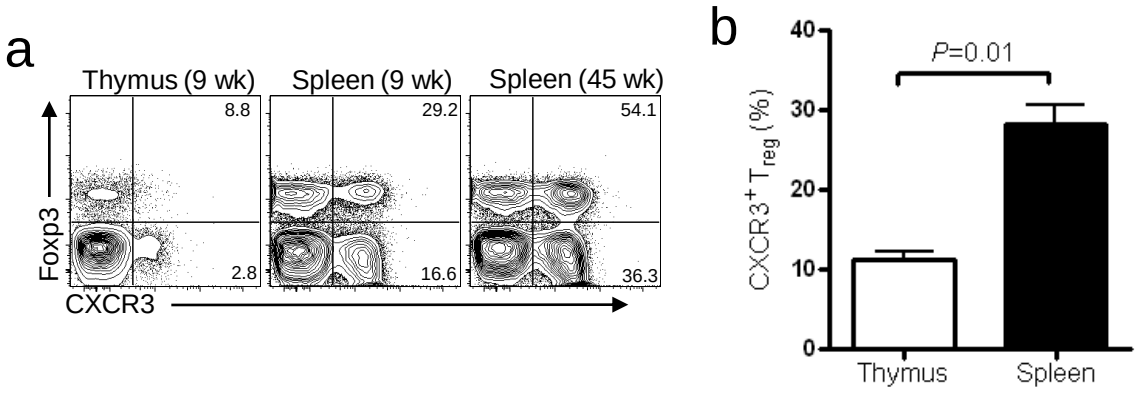


Supplementary Figure 1
***Tbx21*$^{-/-}$ T_{reg} cells express typical T_{reg} cell-associated surface markers and homing receptors.**

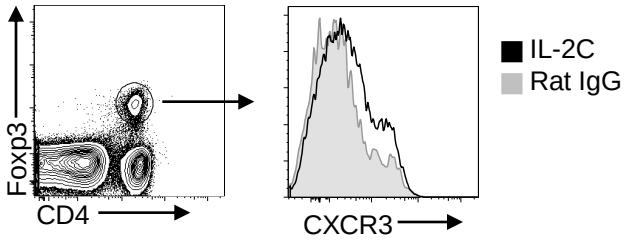
Representative flow cytometry analysis of gated $CD4^+$ splenocytes isolated from *Tbx21*$^{-/-}$ (top) and WT (bottom) mice. Numbers in each plot represent the percent of $CD4^+Foxp3^+$ cells expressing the indicated markers. Data are representative of 6 mice of each genotype analyzed in this fashion.



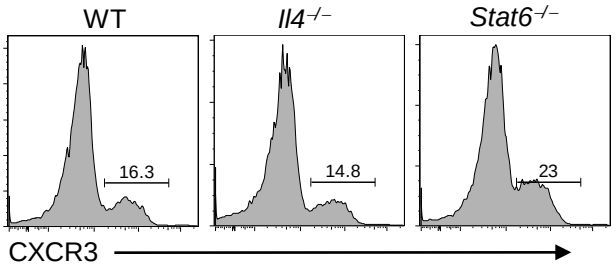
Supplementary Figure 2
T-bet mRNA is enriched in CXCR3$^+$ T$_{reg}$ cells.
qPCR analysis of T-bet mRNA expression in sorted CD4$^+$Foxp3$^+$CXCR3$^-$CD62L$^+$ (Foxp3$^+$CXCR3$^-$), CD4$^+$Foxp3$^+$CXCR3$^+$CD62L$^-$(Foxp3$^+$CXCR3$^+$) and CD4$^+$Foxp3$^-$ CXCR3$^+$CD62L$^-$(Foxp3$^-$CXCR3$^+$) populations isolated from the spleens of Foxp3^{gfp} mice. T-bet mRNA was normalized to β-actin mRNA, and presented in arbitrary units. Data are representative of 4 independent experiments.



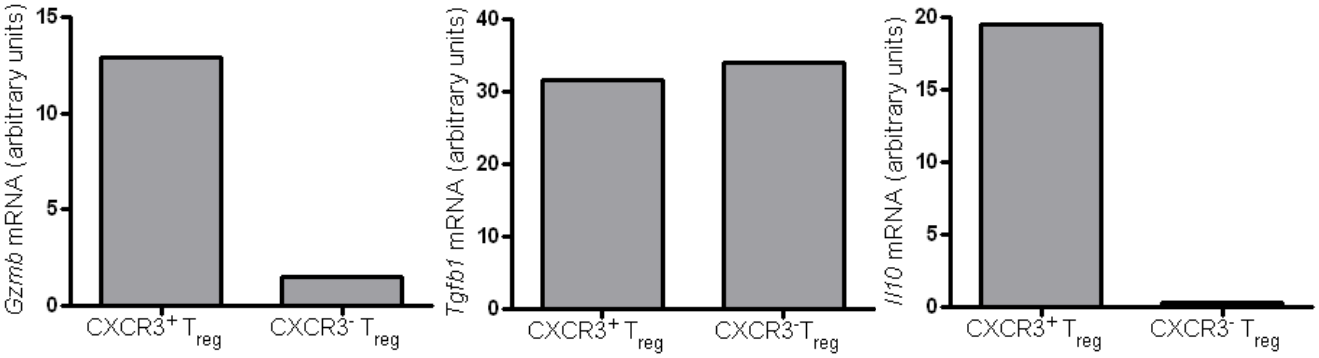
Supplementary Figure 3
Foxp3^{+}CXCR3^{+} T$_{reg}$ cells accumulate with age and are infrequent in the thymus.
(a) Representative flow cytometry analysis of Foxp3 and CXCR3 expression by gated CD4^{+}CD8^{-} cells isolated from the thymus and spleen of 9 week and 45 week old Foxp3^{gfp} mice as indicated. Numbers in plots represent the percent CXCR3^{+} cells among total CD4^{+}Foxp3^{+} cells (top) or CD4^{+}Foxp3^{-} cells (bottom). Data are representative of 3 mice per group analyzed in this fashion.
(b) Mean and s.d. of the frequency of CXCR3^{+} lymphocytes among total CD4^{+}Foxp3^{+} cells recovered from the thymus (white bars) or spleen (black bars) of 9 week old Foxp3^{gfp} mice. *P*-value calculated using a two-tailed, paired student's *t*-test with a 95% confidence interval.



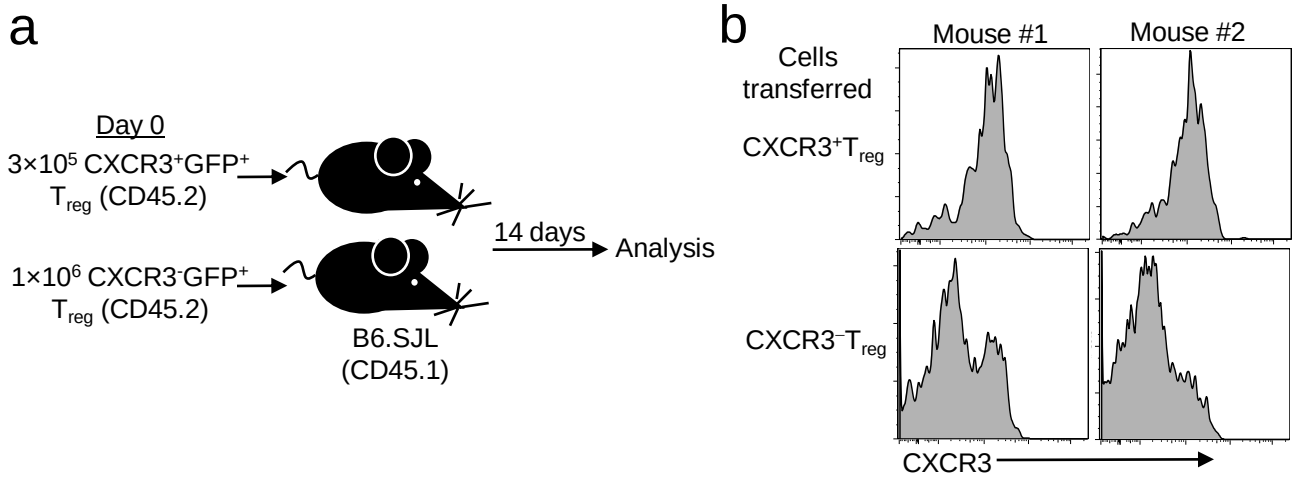
Supplementary Figure 4
IL-2C treatment does not increase the fraction of CXCR3$^+$ T_{reg} cells.
Flow cytometric analysis of CXCR3 expression by gated CD4$^+$Foxp3$^+$ T_{reg} cells isolated from mice treated with IL-2C (open histogram) or rat IgG (shaded histogram). Data are representative of 3 mice per group analyzed in this fashion.



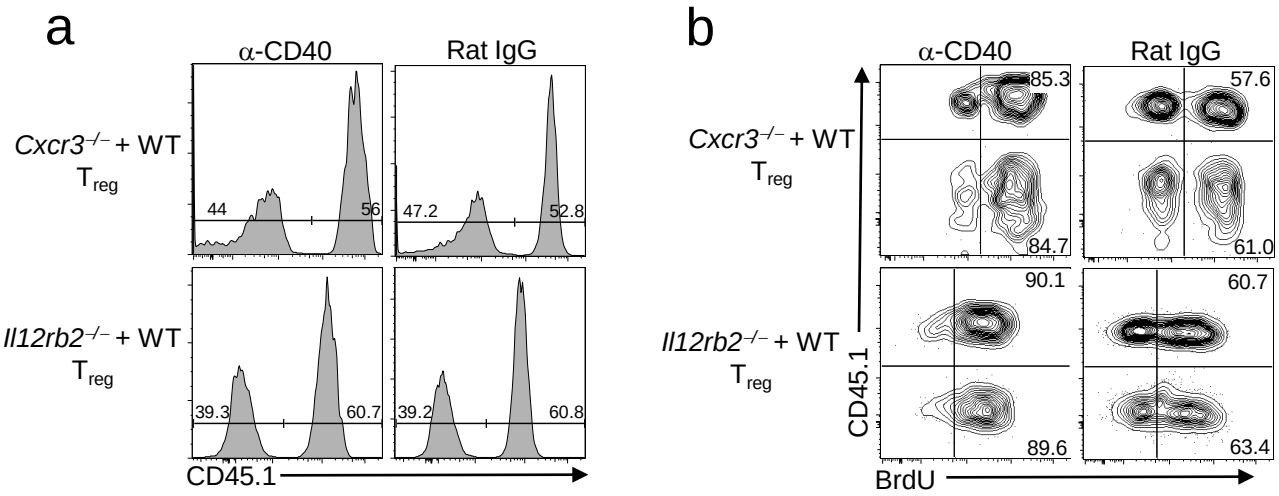
Supplementary Figure 5
IL-4 and STAT6 are dispensable for CXCR3 expression on T_{reg} cells.
Flow cytometric analysis of CXCR3 expression on gated $CD4^+Foxp3^+$ splenocytes isolated from WT, *Il4*$^{-/-}$ and *Stat6*$^{-/-}$ animals as indicated. Numbers display percent of cells positive for CXCR3. Data are representative of greater than 3 mice per genotype analyzed in this fashion.



Supplementary Figure 6
Effector molecule expression by CXCR3$^+$ T_{reg} cells.
Quantitative reverse transcriptase PCR (qPCR) analysis of *Gzmb*, *Tgfb1* and *Il10* mRNA expression in CD4$^+$Foxp3$^+$CXCR3$^+$CD62L$^-$ (CXCR3$^+$$T_{reg}$) and CD4$^+$Foxp3$^+$CXCR3$^-$CD62L$^+$ (CXCR3$^-$$T_{reg}$) cells isolated from the spleens of Foxp3^{gfp} mice. Target mRNA was normalized to β-actin mRNA and presented in arbitrary units. Data are representative of 2 independent experiments.



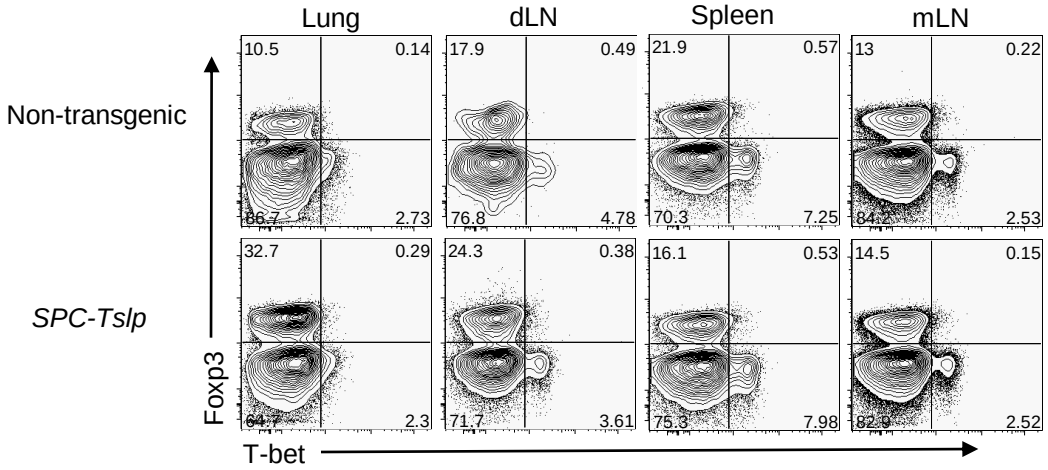
Supplementary Figure 7
CXCR3 expression by T_{reg} cells is stable.
(a) Experimental design. 3×10^5 $CD4^+GFP^+CXCR3^+$ or 1×10^6 $CD4^+GFP^+CXCR3^-$ cells (both $CD45.2^+$) were injected into wild-type mice ($CD45.1^+$). Two weeks post transfer, splenocytes were isolated from recipients and the phenotype of the transferred cells was analyzed by flow cytometry.
(b) Representative flow cytometry analysis of CXCR3 expression on gated $CD4^+Foxp3^+CD45.2^+CD45.1^-$ cells isolated from the spleens of recipient B6.SJL mice. Data are representative of 3 mice or greater per group analyzed in this fashion.



Supplementary Figure 8

CXCR3 and IL-12Rβ2 are dispensable for the accumulation and proliferation of T_{reg} cells following anti-CD40 treatment.

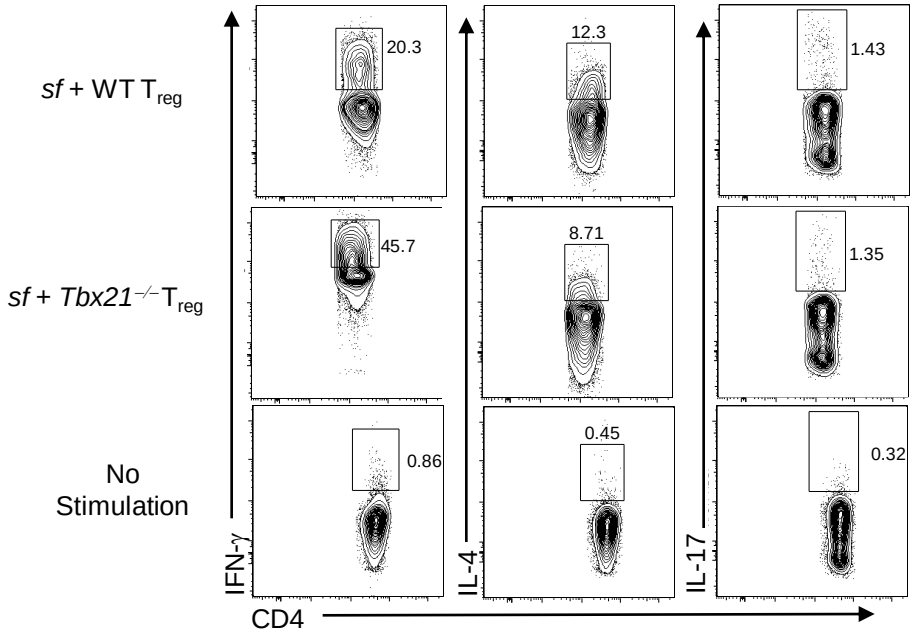
(a) Representative flow cytometry analysis of CD45.1 expression on gated $CD4^+Foxp3^+TCR\beta^+B220^-$ splenocytes isolated from TCRβδ-KO mice treated with the indicated antibodies following adoptive transfer of WT ($CD45.1^+$) and *Cxcr3*$^{-/-}$ ($CD45.1^-$) T_{reg} cells (top) or WT ($CD45.1^+$) and *Il12rb2*$^{-/-}$ ($CD45.1^-$) T_{reg} cells (bottom). Plots are gated on $CD4^+Foxp3^+$ cells. Numbers in histograms indicate the percent of cells positive or negative for CD45.1.
(b) Representative flow cytometry analysis of BrdU incorporation and CD45.1 expression by gated $CD4^+Foxp3^+TCR\beta^+B220^-$ splenocytes isolated from mice described in a. Numbers in FACS plots indicate percentage of $BrdU^+$ cells as a fraction of total WT ($CD45.1^+$) or *Cxcr3*$^{-/-}$ (top, $CD45.1^-$) or *Il12rb2*$^{-/-}$ (bottom, $CD45.1^-$) cells. For **a** and **b**, data are representative of 4 mice analyzed per group.



Supplementary Figure 9

T-bet$^+$ T_{reg} cells do not accumulate during T_H2-mediated inflammatory disease.

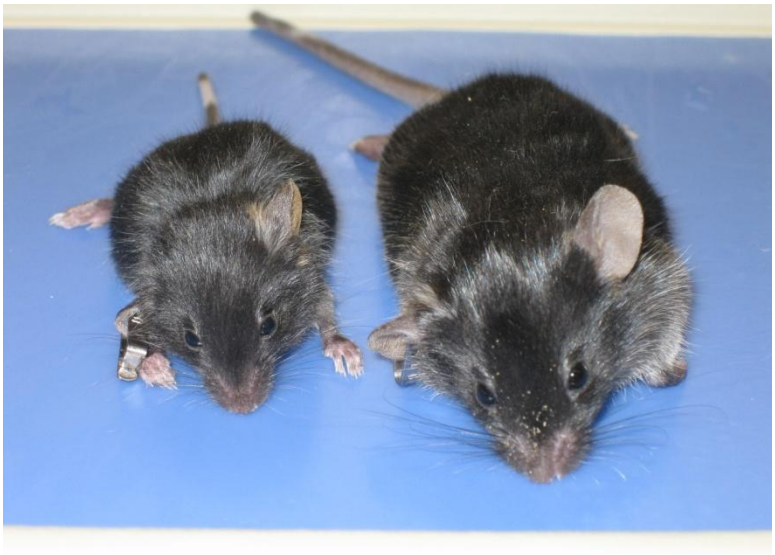
Representative flow cytometry analysis of T-bet and Foxp3 expression by gated CD4$^+$CD8$^-$ lymphocytes isolated from 4.5 month old non-transgenic (top) or *SPC-Tslp* mice (bottom). Numbers in dot plots display the frequency of cells positive for the indicated markers. Data are representative of 3 mice per genotype analyzed in this fashion.



Supplementary Figure 10
Equivalent production of IL-4 and IL-17 by CD4⁺ T cells from *sf* mice given wild-type or *Tbx21*$^{-/-}$ T_{reg} cells.

Representative flow cytometry analysis of pro-inflammatory cytokine production (IFN-γ, IL-4 and IL-17 as indicated) by $CD4^+CD44^{hi}$ effector T cells isolated from *sf* mice given WT (top) or *Tbx21*$^{-/-}$ (middle) T_{reg} cells as neonates following *in vitro* stimulation with PMA and ionomycin. Bottom panels indicate baseline cytokine production by unstimulated $CD4^+CD44^{hi}$ effector cells cultured with monensin for 4 hours. Numbers in plots indicate percent of cytokine-expressing cells as a fraction of total $CD4^+CD8^-CD44^{hi}$ effector T cells. Data are representative of 3 mice per group analyzed in this fashion.

sf + *Tbx21*$^{-/-}$ T_{reg} *sf* + WT T_{reg}



Supplementary Figure 11
Severe runting in *sf* mice given *Tbx21*$^{-/-}$ T_{reg} cells.
Photograph of representative 5 week old *sf* mice given either *Tbx21*$^{-/-}$ (left) or WT (right) T_{reg} cells as neonates. Representative of 10 mice analyzed in 6 independent experiments.

Transferred T_{reg}	0-25 d	26-50 d	>50 d	Cumulative Incidence
WT	0/7*	0/5	0/5	0/7 (0%)
Tbx21$^{-/-}$	2/10	3/10	2/10	7/10 (70%)

Supplementary Table 1
***Tbx21*$^{-/-}$ T_{reg} fail to protect *sf* mice from severe inflammatory disease**
Following transfer of 1×10^6 WT or *Tbx21*$^{-/-}$ T_{reg} cells, *sf* mice were observed for signs of inflammatory disease as evidenced by runting and hunched posture.
* 2 mice receiving WT T_{reg} cells were euthanized before 25 days to use as age-matched controls for diseased recipients of *Tbx21*$^{-/-}$ T_{reg} cells.